Supplementary Information:

In-depth quantitative profiling of post-translational modifications of timothy grass pollen allergome in relation to environmental oxidative stress

Katarina Smiljanic[1], Ivana Prodic[2], Danijela Apostolovic[3], Anka Cvetkovic[4], Djordje Veljovic[5], Jelena Mutic[1,6], Marianne van Hage[3], Lidija Burazer[7], and Tanja Cirkovic Velickovic[1,6,8]*

[1]University of Belgrade – Faculty of Chemistry, Centre of Excellence for Molecular Food Sciences, Belgrade, Serbia, email: katarinas@chem.bg.ac.rs

[2]Innovation Center Ltd, University of Belgrade - Faculty of Chemistry, Belgrade, Serbia

[3]Karolinska Institute, Department of Medicine, Solna, Stockholm, Sweden

[4]Institute of Public Health of Belgrade, Belgrade, Serbia

[5]University of Belgrade – Faculty of Technology and Metallurgy, Belgrade, Serbia

[6]Ghent University Global Campus, Incheon, South Korea

[7]Institute of Immunology, Virology and Sera Production, Torlak, Belgrade, Serbia

[8]Ghent University, Faculty of Bioscience Engineering, Ghent, Belgium

Running Title: Wide and quantitative PTM profiling of multiple source polluted grass pollen

*Corresponding author:

Professor Tanja Cirkovic Velickovic, PhD

University of Belgrade – Faculty of Chemistry

Centre of Excellence for Molecular Food Sciences and Department of Biochemistry

Studentski trg 16, 11 000 Belgrade, Serbia

E-mail 1: tcirkov@chem.bg.ac.rs; E-mail: Tanja.Velickovic@ghent.ac.kr

Tel.: +381 113336608; Fax: +381 112184330;

Materials and Methods

Table S1. Patient demographic and ImmunoCAP values to commercial *Phleum pratense* pollen extract (code=g6).

Patient No	Age	Sex	kU_A/L (g6)		Class
1	32	M	126	high g6 class patients	6
2	41	M	94.7		5
3	49	M	85.6		5
4	33	F	82.6		5
5	36	F	41.04		4
6	46	M	34.2		4
7	39	F	33.7		4
8	34	M	31.5		4
9	38	F	18.1		4
10	33	F	16.1	Moderate to low g6 class patients	3
11	37	M	13.1		3
12	51	F	12.2		3
13	29	M	11.8		3
14	30	M	11.1		3
15	27	F	8.8		2
16	34	F	4.2		1
17	26	M	14.5		3
18	25	F	9.9		2

In gel and in solution digestion for mass spectrometry and shotgun proteomics analysis

After colloidal CBB staining and scanning, 2D gel spots were excised and in-gel digested using the method of Shevchenko et al. [19]. The proteins were digested with proteomics-grade porcine trypsin in a ratio of 1:20 (between 25-75 ng of trypsin in 25 mM ABC depending on protein gel spot quantity). In solution digestion of the short ragweed pollen fractions was done according to "urea" protocol https://masspec.scripps.edu/services/proteomics/insol_prot.php as previously

described [14]. Briefly, 10 µg of pollen protein samples P1 and P2 were reconstituted in 100 µL of 6M urea dissolved in 25 mM ammonium bicarbonate buffer (ABC) pH 8.5. DTT was added to final concentration of 10 mM as reducing reagent (1 h, at RT with agitation). Iodoacetamide was added as alkylating reagent (1 h, dark). Sample was diluted with 25 mM ABC to 1 mL. Trypsin digestion was performed over night at 37 C in ratio 1:30 to approximate amount of protein by weight. Samples were filtered and cleaned with zip-tips C18 (Thermo Fisher Scientific Inc., Bremen, Germany.

Results

Table S2 PAH content in the samples of Phleum pratense pollen determined with GC-MS

µg/kg	sample P1	sample P2
Naphthalene	**104.8±14.9**	**20.4±2.9***
Acenaphthylene	<5.0	**20.9±3.0***
Acenaphthene	<5.0	<5.0
Fluorene	<5.0	<5.0
Phenanthrene	**319.3±39.9**	**171.3±21.4**
Anthracene	<5.0	<5.0
Fluoranthen	<5.0	**14.2±1.8***
Pyrene	<5.0	<5.0
Benzo(a)anthracene	<5.0	<5.0
Chrysene	<5.0	<5.0
Benzo(b)fluoranthene	<5.0	<5.0
Benzo(k)fluoranthene	<5.0	<5.0
Benzo[a] pyrene	<5.0	<5.0
Indeno(1,2,3-cd) pyrene	<5.0	<5.0
Dibenzo(ah)anthracene	<5.0	<5.0
Benzo(ghi)perylene	<5.0	<5.0

* Significantly different compared to sample P1 as determined with two tail unpaired t test.

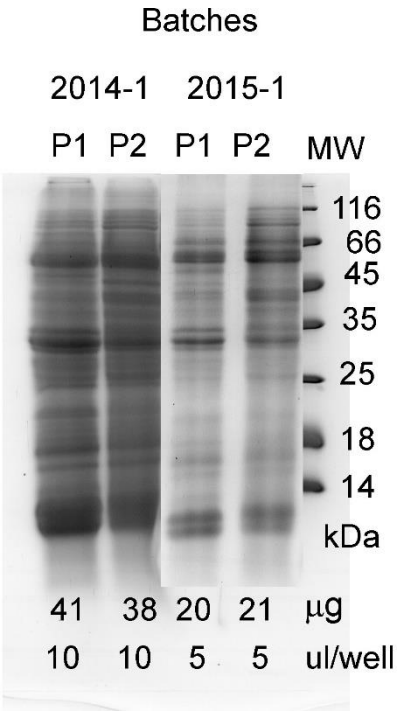

Figure S1 Representative example of *Phlem pratense* pollen protein extracts and their 1D SDS-PAG electroforetic profiles in denaturing conditions from both pollination seasons. P1 - envornmentally preserved; P2 - polluted areas

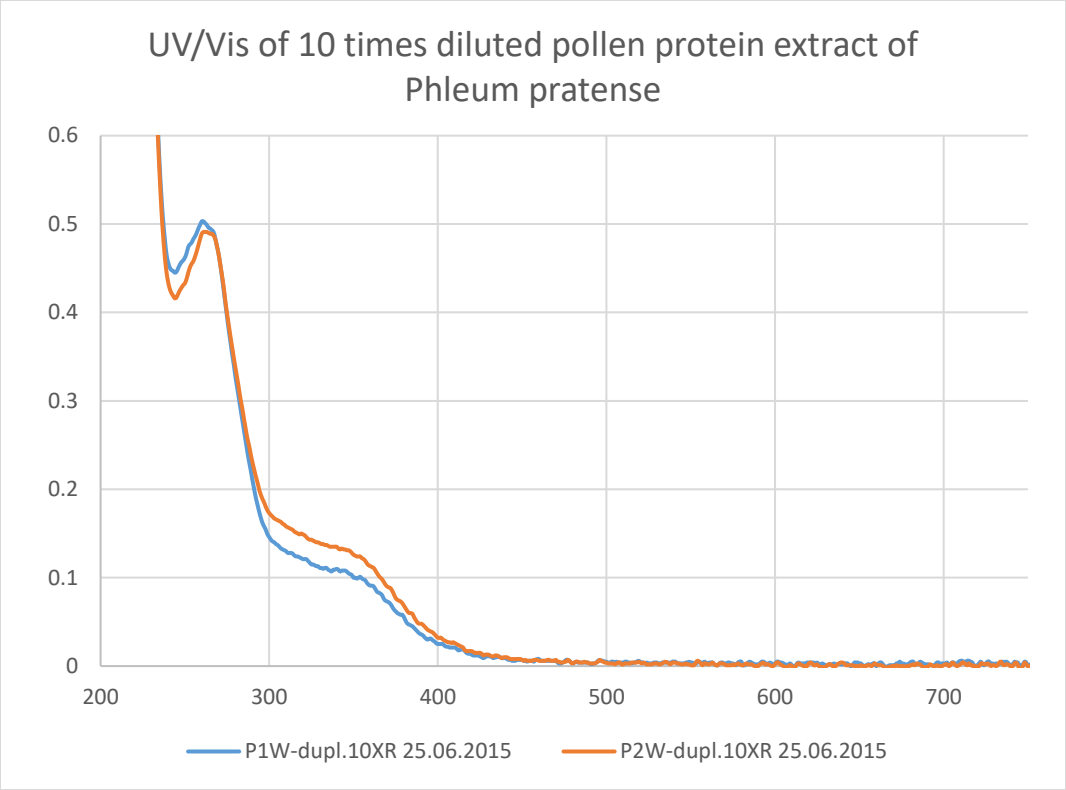

Figure S2 representative example of UV/Vis spectra of 10 times diluted aqueous pollen extract of *Phleum pratense* pollen from envornmentally preserved (P1) and polluted (P2) areas.